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(71) **Applicant** *(for all designated States except US)*: **THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS** [US/US]; 352 Henry Administration Building, 506 Wright, Urbana, IL 61801 (US).

(72) **Inventors; and**

(75) **Inventors/Applicants** *(for US only)*: **LU, Yi** [CN/US]; 2512 Waterville Dr., Champaign, IL 61822 (US). **LIU, Juewen** [CN/US]; 703 E. Colorado Avenue, Apt. 201, Urbana, IL 61801 (US).

(74) **Agent: RAUCH, Paul, E.**; Evan Law Group, Llc, 600 West Jackson, Suite 625, Chicago, IL 60661 (US).

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*[Continued on next page]*

(54) **Title:** APTAMER-BASED COLORIMETRIC SENSOR SYSTEMS
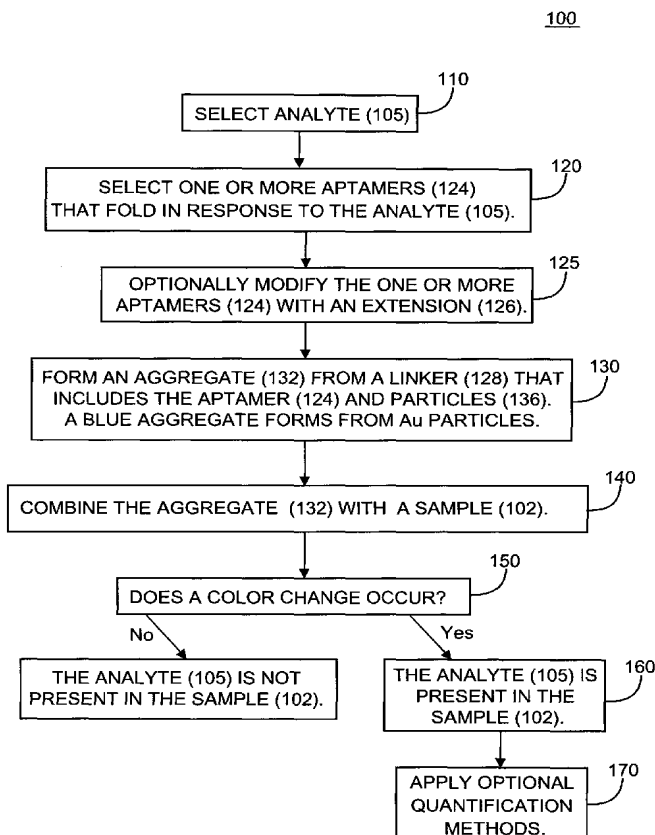
(57) **Abstract:** The present invention provides an aptamer-based colorimetric sensor system for determining the presence and optionally the concentration of an analyte in a sample. Methods of utilizing the sensor system and kits that include the sensor also are provided. The sensor utilizes a linker and oligonucleotide functional ized particles to form an aggregate, which disaggregates in response to the analyte.

# Aptamer-Based Colorimetric Sensor Systems

## FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

## BACKGROUND

[002]      The ability to determine the presence of an analyte in a sample is of significant benefit.  For example, many metals and metal ions, such as lead, mercury, cadmium, chromium, and arsenic, pose significant health risks when present in drinking water supplies.  To prevent the contamination of drinking and other water supplies, it is common to test industrial waste-streams before their release to the water treatment plant.  Biological fluids, such as blood and those originating from body tissues, also may be tested for a variety of analytes to determine if the body has been exposed to harmful agents or if a disease state exists.  For example, the need to detect trace amounts of anthrax in a variety of samples has recently emerged.

[003]      Colorimetric methods are commonly used for the detection of metals and ions in soil, water, waste-streams, biological samples, body fluids, and the like.  In relation to instrument based methods of analysis, such as atomic absorption spectroscopy, colorimetric methods tend to be rapid and require little in the way of equipment or user sophistication.  For example, colorimetric tests are available to aquarists that turn darker shades of pink when added to aqueous samples containing increasing concentrations of the nitrate ($NO_3^-$) ion.  In this manner, colorimetric tests

show that the analyte of interest, such as nitrate, is present in the sample and also may provide an indicator of the amount of analyte in the sample through the specific hue of color generated. While conventional colorimetric tests are extremely useful, they only exist for a limited set of analytes, and often cannot detect very small or trace amounts of the analyte.

[004]     As can be seen from the above description, there is an ongoing need for colorimetric sensor systems that can identify trace amounts of a broader scope of analytes and that increase the reliability of the analysis.

**SUMMARY**

[005]     A sensor system for detecting an analyte includes a linker comprising an aptamer that folds in response to the analyte and second particles coupled to a second oligonucleotide that is complementary to at least a portion of the aptamer. The linker may include an extension where a first oligonucleotide coupled to first particles is complementary to at least a portion of the extension.

[006]     A method of detecting an analyte includes combining an aggregate with a sample to detect a color change responsive to the analyte. The aggregate may include a linker and second particles. The aggregate also may include first particles and the linker may include an extension.

[007]     A kit for detecting an analyte includes a first container containing a system for forming aggregates that includes second particles and a linker including an aptamer, which folds in response to the analyte. The second particles are coupled to second oligonucleotides that are complementary to at least a portion of the aptamer.

[008]     A method for determining the sensitivity and selectivity of an aptamer to an analyte includes combining an aggregate with the analyte, detecting a color change responsive to the analyte, and determining if the DNA strand folded to

provide the color change. The aggregate includes second particles and a linker including a DNA strand. The aggregate also may include first particles. The linker may include an extension.

[009]        In order to provide a clear and consistent understanding of the specification and claims, the following definitions are provided.

[0010]        The term "sample" is defined as a composition that will be subjected to analysis that is suspected of containing the analyte of interest. Typically, a sample for analysis is in a liquid form, and preferably the sample is an aqueous mixture. A sample may be from any source, such as an industrial sample from a waste-stream or a biological sample, such as blood, urine, or saliva. A sample may be a derivative of an industrial or biological sample, such as an extract, a dilution, a filtrate, or a reconstituted precipitate.

[0011]        The term "analyte" is defined as one or more substance potentially present in the sample. The analysis determines the presence, quantity, or concentration of the analyte present in the sample.

[0012]        The term "colorimetric" is defined as an analysis where the reagent or reagents constituting the sensor system produce a color change in the presence or absence of an analyte.

[0013]        The term "light-up" refers to a colorimetric sensor system that undergoes a desired color change in response to an analyte present in a sample.

[0014]        The term "light-down" refers to a colorimetric sensor system that does not undergo a color change when an analyte is present in a sample, but does under a desired color change occurs in the absence of the analyte.

[0015]        The term "sensitivity" refers to the smallest increase in an analyte concentration that is detectable by the sensor system (resolution) or to the lowest concentration limit at which a sensor system can differentiate a signal responsive to the

analyte from a background signal (detection limit). Thus, the more sensitive a sensor system is to an analyte, the better the system is at detecting lower concentrations of the analyte.

[0016]      The term "selectivity" refers to the ability of the sensor system to detect a desired analyte in the presence of other species.

[0017]      The term "hybridization" refers to the ability of a first polynucleotide to form at least one hydrogen bond with at least one second nucleotide under low stringency conditions.

[0018]      The term "aptamer" refers to a strand of nucleic acids that undergoes a conformational change in response to an analyte.

[0019]      The term "conformational change" refers to the process by which an aptamer adopts a tertiary structure from another state. For simplicity, the term "fold" may be substituted for conformational change.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0020]      The invention can be better understood with reference to the following drawings and description. The components in the figures are not necessarily to scale and are not intended to accurately represent molecules or their interactions, emphasis instead being placed upon illustrating the principles of the invention.

[0021]      FIG. 1 represents a colorimetric analysis for determining the presence and optionally the concentration of an analyte in a sample.

[0022]      FIG. 2A depicts an aptamer that depends on two analyte molecules to fold.

[0023]      FIG. 2B depicts the base pairs for an aptamer depending on two adenosine molecules to fold.

[0024]     FIG. 3A represents the disaggregation of an aggregate in the presence of an adenosine analyte.

[0025]     FIG. 3B represents the tail-to-tail hybridization of oligonucleotide functionalized particles with a linker.

[0026]     FIG. 3C represents the head-to-tail hybridization of oligonucleotide functionalized particles with a linker.

[0027]     FIG. 3D represents the head-to-head hybridization of oligonucleotide functionalized particles with a linker.

[0028]     FIG. 4 is a graph relating extinction ratios to the wavelengths of light emitted from a sample by aggregated (solid line) and disaggregated (dashed line) gold nanoparticles.

[0029]     FIG. 5A is a graph showing the change in extinction ratios over time for samples containing guanosine (o), cytidine (▲), uridine (□), and adenosine (•).

[0030]     FIG. 5B is a graph depicting the correlation between the observed extinction ratios for the color change of the sensor system and the concentration of the adenosine analyte after one minute.

[0031]     FIG. 5C is a graph depicting the extinction ratios for multiple adenosine concentrations over a 6 minute time period.

[0032]     FIG. 6A provides the sequences of the extension and aptamer portions of a linker and of the oligonucleotide functionalized particles for a potassium ion sensor system.

[0033]     FIG. 6B depicts the aptamer folding in the presence of the K(I) analyte.

[0034]     FIG. 6C depicts the extinction ratio of the potassium ion sensor for multiple metal ions.

[0035]    FIG. 7A provides the sequences of the extension and aptamer portions of a linker and of the oligonucleotide functionalized particles for a cocaine sensor system.

[0036]    FIG. 7B depicts the aptamer folding in the presence of the cocaine analyte.

[0037]    FIG. 8A is a graph showing the change in extinction ratios over time for samples containing adenosine ($\Delta$), sucrose (o), and cocaine (•).

[0038]    FIG. 8B is a graph depicting the correlation between the observed extinction ratios for the color change of the sensor system and the concentration of the cocaine analyte after 1 minute of aggregation.

[0039]    FIG. 8C is a graph depicting the extinction ratios for multiple cocaine analyte concentrations over a 5 minute time period.

## DETAILED DESCRIPTION

[0040]    Aptamers may be easier to isolate than nucleic acid based catalysts. The simpler structure of aptamers in relation to nucleic acid enzymes also may allow for the design of analyte sensor systems for which nucleic acid enzymes are not available.  The present invention makes use of the discovery that by selecting the hybridization strength between the folded and unfolded conformations of an aptamer and an oligonucleotide functionalized particle, the particle may be released in response to an analyte.  In this manner, a light-up colorimetric sensor is provided that undergoes a desired color change in response to a selected analyte at room temperature, thus overcoming a disadvantage of the sensor system disclosed in U.S. Ser. No. 10/144,679.

[0041]    FIG. 1 represents a colorimetric analysis **100** for determining the presence and optionally the concentration of an analyte **105** in a sample **102**.

In **110**, the analyte **105** for which the method **100** will determine the presence/concentration of is selected.

**[0042]** In one aspect, the analyte **105** may be any ion that causes an aptamer **124** to fold. In another aspect, the analyte **105** may be any metal ion that causes an aptamer **124** to fold. Preferable monovalent ions having a $^+1$ formal oxidation state (I) include $NH_4^+$, K(I), Li(I), Tl(I), and Ag(I). Preferable divalent metal ions having a $^+2$ formal oxidation state (II) include Mg(II), Ca(II), Mn(II), Co(II), Ni(II), Zn(II), Cd(II), Cu(II), Pb(II), Hg(II), Pt(II), Ra(II), Sr(II), Ni(II), and Ba(II). Preferable trivalent and higher metal ions having $^+3$ (III), $^+4$ (IV), $^+5$ (V), or $^+6$ (VI) formal oxidation states include Co(III), Cr(III), Ce(IV), As(V), U(VI), Cr(VI), and lanthanide ions. More preferred analyte ions include monovalent metal ions and metal ions that are toxic to living organisms, such as Ag(I), Pb(II), Hg(II), U(VI), and Cr(VI).

**[0043]** In another aspect, the analyte **105** may be any biomolecule that causes the aptamer **124** to fold. Preferable biomolecules include large biomolecules, such as proteins (e.g. proteins related to HIV, hCG-hormone, insulin), antibodies, growth factors, enzymes, virus (e.g. HIV, small pox), viral derived components (e.g. HIV-derived molecules), bacteria (e.g. anthrax), bacteria derived molecules and components (e.g. anthrax derived molecules), or cells. Preferable biomolecules also may include small biomolecules, such as amino acids (e.g. arginine), nucleotides (e.g. ATP, GTP), neurotransmitters (e.g. dopamine), cofactors (e.g. biotin), peptides, or amino-glycosides.

**[0044]** In another aspect, the analyte **105** may be any organic molecule that causes the aptamer **124** to fold. Preferable organic molecules include drugs, such as antibiotics and theophylline, or controlled substances, such as cocaine, dyes, oligosaccharides, polysaccharides, glucose, nitrogen fertilizers, pesticides, dioxins, phenols, 2,4-dichlorophenoxyacetic acid, nerve gases, trinitrotoluene (TNT), or dinitrotoluene (DNT).

[0045]      Once the analyte **105** is selected, the one or more aptamer **124** is selected that folds in response to the analyte **105**. The aptamer selection **120** may be performed by *in vitro* selection, directed evolution, or other method known to those of ordinary skill in the art. The aptamer selection **120** may provide one or more aptamers that demonstrate enhanced folding in the presence of the selected analyte **105** (thereby providing sensor sensitivity). The selection **120** also may exclude aptamers that fold in the presence of selected analytes, but that do not fold in the presence of non-selected analytes and/or other species present in the sample **102** (thereby providing sensor selectivity).

[0046]      For example, an aptamer may be selected that specifically binds K(I), while not significantly binding Na(I), Li(I), Cs(I), Rb(I), or other competing metal ions. In one aspect, this may be achieved by isolating aptamers that bind K(I), then removing any aptamers that bind Na(I), Li(I), Cs(I), or Rb(I). In another aspect, aptamers that bind Na(I), Li(I), Cs(I), or Rb(I) are first discarded and then those that bind K(I) are isolated. In this manner, the selectivity of the aptamer may be increased.

[0047]      The aptamer **124** includes a nucleic acid strand that folds in the presence of the analyte **105**. In one aspect, the folding may be considered the conversion of a primary or duplex structure to a tertiary structure. The base sequence of the aptamer may be designed so that the aptamer may undergo at least partial hybridization with at least one oligonucleotide functionalized particle. In this aspect, at least a portion of the base sequence of the aptamer **124** may be complementary to at least one oligonucleotide of the oligonucleotide functionalized particle.

[0048]      The aptamer **124** may be formed from deoxyribonucleotides, which may be natural, unnatural, or modified nucleic acids. Peptide nucleic acids (PNAs), which include a polyamide backbone and nucleoside bases (available from Biosearch, Inc., Bedford, MA, for example), also may be useful.

[0049]      Table I below lists analytes, the aptamer or aptamers that bind with and fold in response to that analyte, and the reference or references where the sequence of each aptamer is described.  The analyte binding region of these, and other, aptamers may be adapted for use in a linker **128**.  For example, the non-analyte binding region of the cocaine aptamer, given as SEQ ID NO. 10 in Table I below, may be modified to provide the aptamer GGGAGACAAGGATAAATCCTTCAATGAAGTGGGTCTCCC (SEQ ID NO. 56) and included in the linker **128**.

| Analyte class | Example | Aptamer Motif Sequence (SEQ ID NO.) | Ref |
|---|---|---|---|
| Metal ions | K(I) | GGGTTAGGGTTAGGGTTAGGG <br> (SEQ ID NO. 1) | 1 |
| | Zn(II) | AGGCGAGGUGAAAUGAGCGGUAAU AGCCU <br> (SEQ ID NO. 2) | 2 |
| | Ni(II) | GGGAGAGGAUACUACACGUGAUAG UCAGGGAACAUGACAAACACAGGG ACUUGCGAAAAUCAGUGUUUUGCC AUUGCAUGUAGCAG AAGCUUCCG <br> (SEQ ID NO. 3) | 3 |
| Organic dyes | Cibacron blue | GGGAGAATTCCCGCGGCAGAAGCCC ACCTGGCTTTGAACTCTATGTTATTGG GTGGGGGAAACTTAAGAAAACTACC ACCCTTCAACATTACCGCCCTTCAGCC TGCCAGCGCCCTGCAGCCCGGGAAG CTT <br> (SEQ ID NO. 4) | 4 |
| | Malachite green | GGAUCCCGACUGGCGAGAGCCAGG UAACGA AUGGAUCC <br> (SEQ ID NO. 5) | 5 |
| | Sulforhodamine B | CCGGCCAAGGGTGGGAGGGAGGGG GCCGG <br> (SEQ ID NO. 6) | 6 |

| Small organic molecules | Biotin | AUGGCACCGACCAUAGGCUCGGGU UGCCAGAGGUUCCACACUUUCAUC GAAAAGCCUAUGC (SEQ ID NO. 7) | 7 |
| | Theophylline | GGCGAUACCAGCCGAAAGGCCCUU GGCAGCGUC (SEQ ID NO. 8) | 8 |
| | Adenine | GAUAGGACGAUUAUCGAAAAUCAC CAGAUUGGACCCUGGUUAACGAUC CAUU (SEQ ID NO. 9) | 9 |
| | Cocaine | GGGAGACAAGGATAAATCCTTCAATG AAGTGGGTCGACA (SEQ ID NO. 10) | 10 |
| | Dopamine | GGGAAUUCCGCGUGUGCGCCGCG GAAGAGGGAAUAUAGAGGCCAGCA CAUAGUGAGGCCCUCCUCCC (SEQ ID NO. 11) | 11 |
| Amino acids | Arginine | GGGAGCUCAGAAUAAACGCUCAAG GAGGACCGUGCACUCCUCGAACAU UUCGAGAUGAGACACGGAUCCUGC (SEQ ID NO. 12) | 12 |
| | Citrulline | GACGAGAAGGAGUGCUGGUUAUAC UAGCGGUUAGGUCACUCGUC (SEQ ID NO. 13) | 13 |
| Nucleosides & nucleotides | ATP | ACCTGGGGGAGTATTGCGGAGGAAG GT (SEQ ID NO. 14) | 14 |
| | cAMP | GGAAGAGAUGGCGACUAAAACGAC UUGUCGC (SEQ ID NO. 15) | 15 |
| | GTP | UCUAGCAGUUCAGGUAACCACGUA AGAUACGGGUCUAGA (SEQ ID NO. 16) | 16 |

| | | | |
|---|---|---|---|
| RNA | Guanosine | GGGAGCUCAGAAUAAACGCUCAAC CCGACAGAUCGGCAACGCCNUGUU UUCGACANGAGACACCGAUCCUGC ACCAAAGCUUCC (SEQ ID NO. 17) | 17 |
| | Adenosine | ACCTGGGGGAGTATTGCGGAGGAAG GT (SEQ ID NO. 18) | 18 |
| | TAR-RNA | GCAGTCTCGTCGACACCCAGCAGCG CATGTAACTCCCATACATGTGTGCT GGATCCGACGCAG (SEQ ID NO. 19) | 19 |
| Biological cofactors | CoA | GGGCACGAGCGAAGGGCAUAAGCU GACGAAAGUCAGACAAGACAUGGU GCCC (SEQ ID NO. 20) | 20 |
| | NMN | GGAACCCAACUAGGCGUUUGAGGG GAUUCGGCCACGGUAACAACCCCU C (SEQ ID NO. 21) | 21 |
| | FAD | GGGCAUAAGGUAUUUAAUUCCAUA CAAGUUUACAAGAAAGAUGCA (SEQ ID NO. 22) | 22 |
| | Porphyrin | TAAACTAAATGTGGAGGGTGGGACG GGAAGAAGTTTA (SEQ ID NO. 23) | 23 |
| | Vitamin B12 | CCGGUGCGCAUAACCACCUCAGUG CGAGCAA (SEQ ID NO. 24) | 24 |
| Amino- glycosides | Tobramycin | GGGAGAAUUCCGACCAGAAGCUUU GGUUGUCUUGUACGUUCACUGUU ACGAUUGUGUUAGGUUUAACUACA CUUUGCAAUCGCAUAUGUGCGUCU ACAUGGAUCCUCA (SEQ ID NO. 25) | 25 |

| Oligo-saccharides | Cellobiose | GCGGGGTTGGGCGGGTGGGTTCGC TGGGCAGGGGGCGAGTG (SEQ ID NO. 26) | 26 |
|---|---|---|---|
| Poly-saccharides | Sephadex | UACAGAAUGGGUUGGUAGGCAUAC CUAAUCGAGAAUGAUA (SEQ ID NO. 27) | 27 |
| Antibiotics | Viomycin | GGAGCUCAGCCUUCACUGCAAUGG GCCGCUAGGUUGAUGUGCAGUGA AGUCAGCUGAGGCCCAGGGCUGAA AGGAUCGCCCUCCUCGACUCGUGG CACCACGGUCGGAUCCAC (SEQ ID NO. 28) | 28 |
| | Streptomycin | GGAUCGCAUUUGGACUUCUGCCCA GGGGGCACCACGGUCGGAUCC (SEQ ID NO. 29) | 29 |
| | Tetracycline | GGCCUAAAACAUACCAGAUUUCGA UCUGGAGAGGUGAAGAAUUCGACC ACCUAGGCCGGU (SEQ ID NO. 30) | 30 |
| | Vasopressin | ACGTGAATGATAGACGTATGTCGAGT TGCTGTGTGCGGATGAACGT (SEQ ID NO. 31) | 31 |
| Peptides | Substance P | GGGAGCUGAGAAUAAACGCUCAAG GGCAACGCGGGCACCCCGACAGGU GCAAAACGCACCGACGCCCGGCCG AAGAAGGGGAUUCGACAUGAGGCC CGGAUCCGGC (SEQ ID NO. 32) | 32 |
| Enzymes | HIV Rev Transcriptase | UCCGUUUUCAGUCGGGAAAAACUG (SEQ ID NO. 33) | 33 |
| | Human thrombin | GGTTGGTGTGGTTGG (SEQ ID NO. 34) | 34 |
| Growth factors | VEGF$_{165}$ | GCGGUAGGAAGAAUUGGAAGCGC (SEQ ID NO. 35) | 35 |

| Transcription factors | NF-κB | GGGAUAUCCUCGAGACAUAAGAAA CAAGAUAGAUCCUGAAACUGUUUU AAGGUUGGCCGAUCUUCUGCUCGA GAAUGCAUGAAGCGUUCCAUAUUU UU (SEQ ID NO. 36) | 36 |
|---|---|---|---|
| Antibodies | Human IgE | GGGGCACGTTTATCCGTCCCTCCTAG TGGCGTGCCCC (SEQ ID NO. 37) | 37 |
| Gene Regulatory factors | Elongation factor Tu | GGGGCUAUUGUGACUCAGCGGUU CGACCCCGCUUAGCUCCACCA (SEQ ID NO. 38) | 38 |
| Cell adhesion molecules | Human CD4 | UGACGUCCUUAGAAUUGCGCAUUC CUCACACAGGAUCUU (SEQ ID NO. 39) | 39 |
| cells | YPEN-1 endothelial | ATACCAGCTTATTCAATTAGGCGGTG CATTGTGGTTGGTAGTATACATGAGG TTTGGTTGAGACTAGTCGCAAGATAT AGATAGTAAGTGCAATCT (SEQ ID NO. 40) | 40 |
| Viral/bacterial components | Anthrax spores Rous sarcoma virus | Sequences are not given AGGACCCUCGAGGGAGGUUGCGCA GGGU (SEQ ID NO. 42) | 41 42 |

**Table I**

[0050]    <u>Reference Listing for Table I</u>

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|----|------------------------------------------------------------------------------------------------------------------------------------------------------|

[0051]     After selecting an appropriate aptamer or aptamers in **120**, a linker **128** is formed that includes the aptamer **124**. In one aspect, the aptamer **124** may serve directly as the linker **128**. In another aspect, the linker **128** may be formed by joining the aptamer **124** with one or more extensions **126**.

[0052]     The extension **126** may be any nucleic acid sequence that may be joined with the aptamer **124**, that may undergo at least partial hybridization with one or more oligonucleotide functionalized particles, and that is compatible with the analysis **100**. In this aspect, at least a portion of the base sequence of the extension **126** may be complementary to at least one oligonucleotide of one or more oligonucleotide functionalized particle. In one aspect, solid phase synthesis may be used to join the aptamer **124** with the extension **126** to form the linker **128**. In another aspect, after the aptamer **124** portion of the linker **128** is synthesized, the synthesis is continued to form the extension **126**. Similarly, the linker **128** may be extended with the aptamer **124** sequence.

[0053]     Preferably, the extension **126** includes from 1 to 100 bases. In one aspect, at least 50, 70, or 90 % of the bases present in the extension **126** are capable of hybridizing with a complementary portion of a first oligonucleotide functionalized particle, such as the TGAGTAGACACT-5′ (SEQ ID NO. 43) portion of particle **336** in FIG. 3A, while at least 50, 35, 25, or 10 % of the bases present in the extension are capable of hybridizing with a second oligonucleotide functionalized particle, such as particle **337** in FIG. 3A.

[0054]     After selecting or synthesizing the linker **128**, an aggregate **132** may be formed in **130**. The aggregate **132** includes the linker **128** and oligonucleotide functionalized particles **136**. Considering the physical size of its components, the aggregate **132** may be quite large.

[0055]      The linker **128** hybridizes with the oligonucleotide functionalized

particles **136** and includes the aptamer **124** and may include the extension **126**.

For example, if first and second oligonucleotide functionalized particles have base

sequences of 3'-AAAAAAAAAAAATGAGTAGACACT (SEQ ID NO. 44) and

5'-CCCAGGTTCTCT (SEQ ID NO. 45), respectively, an appropriate sequence for the

linker **128** that includes the aptamer **124** that folds in the presence of an adenosine

analyte and the extension **126** may be

5'-ACTCATCTGTGAAGAGAACCTGGGGGAGTATTGCGGAGGAAGGT (SEQ ID

NO. 46).

[0056]      For the adenosine analyte, the extension **126** portion of the linker **128**

is the ACTCATCTGTGAAGAGA (SEQ ID NO. 47) portion of the sequence, which

allows the extension **126** to hybridize with twelve bases of the first functionalized

particle and five bases of the second functionalized particle.  Similarly, the aptamer

**124** portion of the linker **128** is the ACCTGGGGGAGTATTGCGGAGGAAGGT

(SEQ ID NO. 18) portion of the sequence, which allows the ACCTGGG (SEQ ID

NO: 48) portion of the aptamer **124** to hybridize with the TGGACCC (SEQ ID NO.

49) portion of the second functionalized particle.

[0057]      Because the particles **136** demonstrate distance-dependent optical

properties, the particles are one color when closely held in the aggregate **132** and

undergo a color change as the distance between the particles increases.

For example, when the particles **136** are gold nanoparticles, the aggregate **132**

displays a blue color in aqueous solution that turns red as disaggregation proceeds.

[0058]      Disaggregation occurs when the aptamer **124** portion of the linker **128**

binds with and folds in response to the analyte **105**.  When the aptamer **124** folds, a

portion of the hybridization with the second oligonucleotide functionalized particles

is lost.  This hybridization loss allows the second oligonucleotide functionalized

particles to separate from the aggregate **132**.  Thus, as the particles **136** diffuse away

from the aggregate **132**, the solution changes from blue to red.

[0059]        The particles **136** may be any species that demonstrate distance-dependent optical properties and are compatible with the operation of the sensor system.  Suitable particles may include metals, such as gold, silver, copper, and platinum; semiconductors, such as CdSe, CdS, and CdS or CdSe coated with ZnS; and magnetic colloidal materials, such as those described in Josephson, Lee, et al., *Angewandte Chemie,* International Edition (2001), 40(17), 3204-3206.  Specific useful particles may include ZnS, ZnO, $TiO_2$, AgI, AgBr, $HgI_2$, PbS, PbSe, ZnTe, CdTe, $In_2S_3$, $In_2Se_3$, $Cd_3P_2$, $Cd_3As_2$, InAs, and GaAs.

[0060]        In a preferred aspect, the particles are gold (Au) nanoparticles and have an average diameter from 5 to 70 nanometers (nm) or from 10 to 50 nm.  In a more preferred aspect, gold nanoparticles having an average diameter of from 10 to 15 nm are functionalized to the oligonucleotides.

[0061]        For a more detailed treatment of how to prepare oligonucleotide functionalized gold particles, *See* U.S.P. No. 6,361,944; Mirkin, et al., *Nature (London)* **1996**, *382*, 607-609; Storhoff, et al., *J. Am. Chem. Soc.* **1998**, 20, 1959-1064; and Storhoff, et al., *Chem. Rev. (Washington, D. C.)* **1999**, *99*, 1849-1862. While gold nanoparticles are presently preferred, other species that undergo a distance-dependent color change, such as inorganic crystals, quantum dots, and the like also may be attached to oligonucleotides.

[0062]        In **140** the aggregate **132** may be combined with the sample **102**. In **150** the sample **102** is monitored for a color change.  If a color change does not occur, then the analyte **105** is not present in the sample **102**.  If a color change does occur in **160**, the analyte **105** is present in the sample **102**.  The color change signifies that the analyte **105** caused the aptamer **124** to fold, thus allowing the particles **136** to diffuse away from the aggregate **132** and into the solution of the sample **102**. Thus, the analysis **100** provides a "light-up" sensor system because a color change occurs in the presence of the analyte **105**.

[0063]      The rate at which a substantially complete color change occurs in response to the analyte **105** may be considered the response time of the sensor system. In one aspect, the color change may be considered substantially complete when the absorption peak in the visible region increases by 20%. In another aspect, the color change may be considered substantially complete when the extinction coefficient at 522 nm over 700 nm increases by 100% for gold particles. Preferable response times for the sensor system are from 1 second to 60 minutes or from 2 seconds to 10 minutes. More preferable response times for the sensor system are from 5 seconds to 2 minutes or from 8 to 12 seconds. Preferable temperature ranges for operation of the sensor system are from 0° to 60° or from 15° to 40° C. More preferable ranges for operation of the sensor system are from 23° to 37° or from 25° to 30° C. In another aspect, when the analysis **100** is conducted from 23° to 37° C, a preferable response time may be less than 2 minutes or from 1 to 12 seconds.

[0064]      The degree the color changes in response to the analyte **105** may be quantified by colorimetric quantification methods known to those of ordinary skill in the art in **170**. Various color comparator wheels, such as those available from Hach Co., Loveland, CO or LaMotte Co., Chestertown, MD may be adapted for use with the present invention. Standards containing known amounts of the selected analyte may be analyzed in addition to the sample to increase the accuracy of the comparison. If higher precision is desired, various types of spectrophotometers may be used to plot a Beer's curve in the desired concentration range. The color of the sample may then be compared with the curve and the concentration of the analyte present in the sample determined. Suitable spectrophotometers include the Hewlett-Packard 8453 and the Bausch & Lomb Spec-20.

[0065]      In yet another aspect, the method **100** may be modified to determine the sensitivity and selectivity of an aptamer to the analyte **105**. In this aspect, one or more DNA strand suspected of being an aptamer responsive to the analyte **105** is

selected in **120**. The DNA strand may or may not be modified with the extension **126** in **125**. In **130**, an aggregate is formed from the DNA strands and appropriate particles. In **140**, the aggregates are combined with the analyte **105**. If the aggregate undergoes a color change, then the DNA strand is an appropriate aptamer sequence for the target **105**. In this manner, multiple aptamers selected in **120** may be tested for use in a colorimetric sensor system.

[0066]     FIG. 2A depicts an aptamer **224** that depends on two analyte molecules to undergo a conformational change to adopt a folded structure **230**. FIG. 2B depicts the specific base pairs of the aptamer **224** that forms the folded structure **230** in response to two adenosine molecules. While this specific aptamer sequence relies on two molecules of the adenosine analyte to fold, other aptamers may fold in response to a single analyte molecule.

[0067]     FIG. 2C depicts the aptamer **224** joined to an extension **226** to form a linker **228**. The extension **226** includes A and B portions, where the A portion includes enough complementary bases to form stable hybridization with the oligonucleotide of a first particle **236**. For example, a non-complementary poly-adenine chain ($A_{12}$) including 12 adenine bases may be appended to the complementary sequence of the first particle **236** to enhance hybridization stability. The sequence for the B portion of the extension **226** may be selected to eliminate linkers that inherently form stable secondary structures. A computer program, such as M-fold available at (http://www.bioinfo.rpi.edu/applications/mfold/) (M. Zuker. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31 (13), 3406-15, (2003)) or others, may be used to predict stable linkers for elimination. For example if a stable sequence for a linker is ACTCATCTGTGAAGA_TG_ACCTGGGGGAGTATTGCGGAGGAAGGT (SEQ ID NO. 50), by substituting the underlined TG bases for GA bases to give the sequence ACTCATCTGTGAAGA_GA_ACCTGGGGGAGTATTGCGGAGGAAGGT (SEQ ID NO. 51), the linker may be rendered inherently unstable.

[0068]     The aptamer **224** includes C and D portions.  In one aspect, the hybridization stability of the combined B portion of the extension **226** and the C portion of the aptamer **224** with the second particle **237** may be less than that for A and the first particle **236**.  In a preferred aspect, the melting temperature of this C+B/second particle hybridization is higher than the temperature at which the sensor system is to be used.  In another preferred aspect, the melting temperature of a B portion hybridized to the oligonucleotide sequence of the second particle **237** is less than the temperature at which the sensor system is to be used.  In another preferred aspect, the stability of a C+D+analyte complex should be greater than the hybridization stability of C with the second particle **237**.  In another aspect, the sequences of B and C are as short as is compatible with the operation of the sensor system.

[0069]     FIG. 3A depicts the disaggregation of an aggregate **332** in the presence of an adenosine analyte **305**.  The aggregate **332** is formed from multiple aggregate units **331**.  Each of the aggregate units **331** is formed from a linker **328**, which is hybridized to the 3′ and 5′ thiol-oligonucleotide functionalized particles **336** and **337**, respectively.  The linker **328** includes an aptamer portion **324** and an extension portion **326**.  The 3′-$A_{12}Ade_{Au}$ particle **336** hybridizes with the extension **326**, while the 5′-$Ade_{Au}$ particle **337** hybridizes with the extension **326** and the aptamer **324** to from the aggregate unit **331**.

[0070]     While one base sequence for the linker and the particles are shown, the bases may be changed on the opposing strands to maintain the pairings. For example, any cytosine in the A or B portions of the linker **228** may be changed to thymine, as long as the paired base of the particle oligonucleotide is changed from guanine to adenine.

[0071]     In the presence of the adenosine analyte **305**, the aptamer portion **324** of the linker **328** folds, thus eliminating at least a portion of the hybridization between the aptamer portion **324** of the linker **328** and the 5′-$Ade_{Au}$ particle **337**.

This loss of hybridization between the aptamer **324** and the 5'-Ade<sub>Au</sub> particle **337** releases the 5'-Ade<sub>Au</sub> particle to the solution.

[0072]    As the 5'-Ade<sub>Au</sub> particles **337** are released, the blue aggregate **332** begins to disaggregate to form partial aggregate **390**. This partial disaggregation adds red color to the blue solution as the particles **337** diffuse away from the aggregate **332**, thus giving a purple solution. If enough of the adenosine analyte **305** is present in the sample, the reaction will continue until the aggregate **332** is completely disaggregated, to give **395**. Complete disaggregation results in a red solution due to the greater distance between the particles **336, 337**.

[0073]    The alignment of the particles **336, 337** (tail-to-tail, head-to-tail, or head-to-head) with respect to each other may influence how tightly the aggregate units **331** bind. FIG. 3B depicts the aggregate unit **331** formed when the functionalized particles, such as **336** and **337**, hybridize to the linker **328** in a tail-to-tail arrangement. Head-to-tail (FIG. 3C) or head-to-head (FIG. 3D) hybridization may be selected by reversing one or both ends of the oligonucleotide to which the particle is attached, respectively. Thus, by reversing the 3' attachment of functionalized particle **336** to 5', particle **338** may hybridize to give the head-to-tail alignment of FIG. 3C. Similarly by reversing the 3' attachment of the functionalized particle **336** to the 5' attachment of the particle **338** and by reversing the 5' attachment of the particle **337** to the 3' attachment of particle **339**, the particles **338, 339** may hybridize to give the head-to-head alignment of FIG. 3D.

[0074]    At present, the tail-to-tail hybridization arrangement of FIG. 3B is preferred because the head-to-tail and head-to-head hybridization arrangements of FIGs. 3C and 3D may produce aggregates that sterically hinder aptamer binding and/or aggregate formation. However, this steric hindrance may be reduced through a reduction in the average diameter of the particles or by increasing the number of bases functionalized to the particles and the length of the extension, for example.

[0075]     While not shown, the methodology of FIG. 3A may be applied to other analytes, including potassium ions, cocaine, and the analytes listed above in Table I. Table II, below, gives the base sequences of the linkers and particles for adenosine, K(I), and cocaine sensor systems. The aptamer portion of each linker is presented in uppercase, while the extension portion of each linker is presented in lowercase.

| Name | Sequence | SEQ ID NO. |
|------|----------|------------|
| Adenosine Linker | 5'-actcatctgtgaagagaACCTGGGGGAGTATTGCGGAGGAAGGT | 46 |
| 3'-A$_{12}$Ade$_{Au}$ | 3'-AAAAAAAAAAAATGAGTAGACACT | 44 |
| 5'-Ade$_{Au}$ | 5'-CCCAGGTTCTCT | 45 |
| Potassium Linker | 5'-actcatctgtgatctaaGGGTTAGGGTTAGGGTTAGGG | 52 |
| 3'-A$_{12}$K(I)$_{Au}$ | 3'-AAAAAAAAAAAATGAGTAGACACT | 44 |
| 5'-K(I)$_{Au}$ | 5'-AACCCTTAGA | 53 |
| Cocaine Linker | 5'-actcatctgtgaatctc GGGAGACAAGGATAAATCCTTCAATGAAGTGGGTCTCCC | 54 |
| 3'- A$_{12}$CoC$_{Au}$ | 3'-AAAAAAAAAAAATGAGTAGACACT | 44 |
| 5'-CoC$_{Au}$ | 5'-GTCTCCCGAGA | 55 |

**Table II**

[0076]     The ionic strength of the sample may influence how tightly the moieties that form the aggregate bind together. Higher salt concentrations favor aggregation, thus slowing sensor response, while lower salt concentrations may lack the ionic strength necessary to maintain the aggregates. In one aspect, the sample may include or be modified with a reagent to include a monovalent metal ion concentration of 30 mM and greater. The ionic strength of the sample may be modified with Na$^+$ ions, for example. In a preferred aspect, the monovalent metal ion concentration of the sample, which contains the aggregate, is from 150 to 400 mM. At present, especially preferred monovalent metal ion concentrations are

about 300 mM for adenosine and potassium analytes and about 150 mM for cocaine as an analyte. pH also may influence the aggregate binding, possibly attributable to the protonation of the polynucleotide base pairs at lower pH. In one aspect, a pH from 5 to 9 is preferred, with an approximately neutral pH being more preferred.

[0077]     Thus, the performance of the sensor may be improved by adjusting the ionic strength and pH of the sample prior to combining it with the aggregate. Depending on the sample, it may be preferable to add the sample or analyte to a solution containing the aggregate (where the ionic strength and pH may be controlled) or the reverse.

[0078]     The sensor system, including the aptamer, extension, and oligonucleotide functionalized particles may be provided in the form of a kit. In one aspect, the kit includes the aptamer and the extension joined to form the linker. In yet another aspect, the kit includes the extension, but excludes the aptamer, which is then provided by the user or provided separately. In this aspect, the kit also may include the reagents required to link the supplied extension with an aptamer. In this aspect, the kit also may be used to determine the specificity and/or selectivity of various aptamers to a selected analyte. Thus, the kit may be used to select an appropriate aptamer in addition to detecting the analyte. In yet another aspect, the kit includes an exterior package that encloses a linker and oligonucleotide functionalized particles.

[0079]     One or more of these kit components may be separated into individual containers, or they may be provided in their aggregated state. If separated, the aggregate may be formed before introducing the sample. Additional buffers and/or pH modifiers may be provided in the kit to adjust the ionic strength and/or pH of the sample.

[0080]     The containers may take the form of bottles, tubs, sachets, envelopes, tubes, ampoules, and the like, which may be formed in part or in whole from

plastic, glass, paper, foil, MYLAR®, wax, and the like. The containers may be equipped with fully or partially detachable lids that may initially be part of the containers or may be affixed to the containers by mechanical, adhesive, or other means. The containers also may be equipped with stoppers, allowing access to the contents by syringe needle. In one aspect, the exterior package may be made of paper or plastic, while the containers are glass ampoules.

[0081]      The exterior package may include instructions regarding the use of the components. Color comparators; standard analyte solutions, such as a 10 $\mu$m solution of the analyte; and visualization aids, such as thin layer chromatography (TLC) plates, test tubes, and cuvettes, also may be included. Containers having two or more compartments separated by a membrane that may be removed to allow mixing may be included. The exterior package also may include filters and dilution reagents that allow preparation of the sample for analysis.

[0082]      In another aspect, in addition to the sensor system of the present invention, the kit also may include multiple sensor systems to further increase the reliability of analyte determination and reduce the probability of user error. In one aspect, multiple light-up sensor systems in accord with the present invention may be included. In another aspect, a "light-down" sensor system may be included with the light-up sensor system of the present invention. Suitable light-down sensors for inclusion in the presently claimed kit may rely on DNAzyme/Substrate/particle aggregates that are not formed in the presence of the selected analyte. A more detailed description of suitable light-down sensor systems for inclusion in the presently claimed kit may be found, for example, in U.S. Pat. App. 10/756,825, filed January 13, 2004, entitled "Biosensors Based on Directed Assembly of Particles," which is hereby incorporated by reference.

[0083]      The preceding description is not intended to limit the scope of the invention to the described embodiments, but rather to enable a person of ordinary skill in the art to make and use the invention. Similarly, the examples below are not

to be construed as limiting the scope of the appended claims or their equivalents, and are provided solely for illustration. It is to be understood that numerous variations can be made to the procedures below, which lie within the scope of the appended claims and their equivalents.

## EXAMPLES

[0084]      All DNA samples were purchased from Integrated DNA Technology Inc., Coralville, IA. The DNA samples that formed the extensions were purified by gel electrophoresis, while the thiol-modified DNA samples for forming the oligonucleotide functionalized particles were purified by standard desalting. Adenosine, cytidine, uridine, guanosine and cocaine were purchased from Aldrich. Gold nanoparticles having an average diameter of 13 nm were prepared and functionalized with 12-mer thiol-modified DNA following literature procedures, such as those disclosed in Storhoff, J., et al., "One-pot colorimetric differentiation of polynucleotides with single base imperfections using gold particle probes," *JACS* 120: 1959-1964 (1998), for example. The average diameter of the gold nanoparticles was verified by transmission electronic microscope (JEOL 2010).

[0085]      Example 1: Preparation of a Colorimetric Adenosine Sensor

[0086]      Five-hundred microliters of 5'-Ade$_{Au}$ (extinction at 522 nm equals 2.2) and 500 µL of 3'-A$_{12}$Ade$_{Au}$ (extinction at 522 nm equals 2.2) were mixed in the presence of 300 mM NaCl, 25 mM Tris acetate buffer, pH 8.2, and 100 nM of the adenosine aptamer/extension (Adenosine Linker). The sample was warmed to 65° C for one minute and then allowed to cool slowly to 4° C. The nanoparticles changed color from red to dark purple during this process. The sample was centrifuged and the precipitates were collected and then dispersed in 2000 µL of the same buffer (300 mM NaCl, 25 mM Tris acetate, pH 8.2). The suspension was used for detection of adenosine.

[0087]      Example 2:  Colorimetric Detection of Adenosine

[0088]      One-hundred microliters of the sensor suspension from Example 1 was added to a small volume of concentrated adenosine solution.  For example, 2 μL of 50 mM adenosine was added to give a final concentration of 1 mM.  The color change was monitored with a UV-vis spectrometer or by the naked eye.

[0089]      Example 3:  Monitoring the Performance of the Adenosine Sensor

[0090]      The color change of the sample from Example 2 was monitored by UV-vis extinction spectroscopy.  FIG. 4 is a graph relating the extinction ratios provided at specific wavelengths from a sample during disaggregation.  The dashed line in FIG. 4 shows the strong extinction peak at 522 nm exhibited by separated 13 nm nanoparticles, which provide a deep red color.  As may be seen from the solid line in FIG. 4, upon aggregation, the 522 nm peak decreases in intensity and shifts to longer wavelength, while the extinction at 700 nm region increases, resulting in a red-to-blue color transition.  Therefore a higher extinction ratio at 522 to 700 nm is associated with the red color of separated nanoparticles, while a low extinction ratio is associated with the blue color of aggregated nanoparticles.  This extinction ratio was used to monitor the aggregation state of the particles.

[0091]      Example 4:  Selectivity and Sensitivity of the Adenosine Sensor

[0092]      To a quarts UV-vis spectrophotometer cell (Hellma, Germany) was added 100 μL of the adenosine sensor system prepared in Example 1. A small volume (1-5 μL) of solutions including adenosine, uridine, cytidine or guanosine was added to the spectrophotometer cell to bring the analyte concentration to the desired level in the cell.

[0093]      The dispersion kinetics for each cell was monitored as a function of time using a Hewlett-Packard 8453 spectrophotometer.  FIG. 5A is a graph depicting the ratios of extinction at 522 and 700 nm plotted as a function of time.  As may be

seen from the plots, only adenosine gave significant increase in the extinction ratio as a function of time, while uridine, cytidine, and guanosine provided a color change consistent with the background. Therefore, the high selectivity of the sensor was confirmed.

[0094]     FIG. 5B is a graph depicting the correlation between the observed extinction ratios for the color change of the sensor system and the concentration of the adenosine analyte after five minutes of aggregation. The exceptional linearity of the sensor system was evident from about 0.1 to about 1.5 mM.

[0095]     FIG. 5C is a graph depicting the extinction ratios for multiple adenosine analyte concentrations over a 6 minute time period. The graph demonstrates the ability of the sensor system to effectively differentiate between different analyte concentrations within a few minutes. Thus, the ability of the sensor system to provide accurate quantitative information was established.

[0096]     In addition to the instrumental method of FIG. 5B, the color developed by the sensor was conveniently observed visually. A color progression from blue to red was seen as the adenosine concentration increased from 0 to 2 mM. Uridine, cytidine, and guanos provided a color similar to the background.

[0097]     Example 5: Preparation of a Colorimetric Potassium Ion Sensor

[0098]     FIGs. 6A-6B represent an analyte sensor system that includes an aptamer that folds in the presence of K(I). FIG. 6A provides the sequences of the extension and aptamer portions of a linker and of the oligonucleotide functionalized particles. FIG. 6B depicts the aptamer folding in the presence of the K(I) analyte. To prepare the K(I) sensor system, 500 µL of 5'-K(I)$_{Au}$ (extinction at 522 nm equals 2.2) and 500 µL of 3'-A$_{12}$K(I)$_{Au}$ (extinction at 522 nm equals 2.2) were mixed in the presence of 300 mM NaCl, 25 mM Tris acetate buffer, pH 8.2, and 100 nM of the potassium ion aptamer/extension (Potassium Linker). The sample was warmed to 65° C for one minute and then allowed to cool slowly to 4° C. The nanoparticles

changed color from red to dark purple during this process. The sample was centrifuged and the precipitates were collected and dispersed in 10 μL of the same buffer (300 mM NaCl, 25 mM Tris acetate, pH 8.2). The suspension was used for detection of $K^+$.

[0099]        Example 6: Colorimetric Detection of Potassium

[00100]        Metal ion solutions of $Li^+$, $Na^+$, $K^+$, $Rb^+$, or $Cs^+$ ions were made by dissolving LiCl, NaCl, KCl, RbCl or CsCl salt, respectively, in deionized water to obtain an ion concentration of 3 M. From these metal ion stock solutions were prepared solutions containing 25 mM of Tris acetate buffer, pH 8.2, and 300 mM of $Li^+$, $Na^+$, $K^+$, $Rb^+$, or $Cs^+$ ions. To each of these five solutions was added 1 μL of the K(I) sensor system from Example 5 for each 99 μL of the metal ion containing solution. Therefore, each solution contained ~ 300 mM of $Li^+$, $Na^+$, $K^+$, $Rb^+$, or $Cs^+$ metal ions and an additional 3 mM of $Na^+$ ions as background. Each sample was heated to 65° C and then cooled slowly to 4° C in 1 hour. The color change was monitored with a UV-vis spectrometer or by the naked eye. FIG. 6C depicts the extinction ratio of the potassium ion sensor system in the presence of $Li^+$, $Na^+$, $K^+$, $Rb^+$, or $Cs^+$ ions, thus confirming the selectivity of the sensor system to K(I).

[00101]        Example 7: Preparation of a Colorimetric Cocaine Sensor

[00102]        FIGs. 7A-7B represent an analyte sensor system that includes an aptamer that folds in the presence of cocaine. FIG. 7A provides the sequences of the extension and aptamer portions of the linker and of the oligonucleotide functionalized particles. FIG. 7B depicts the aptamer folding in the presence of the cocaine analyte. To prepare the cocaine sensor system, 500 μL of 5'-Coc$_{Au}$ (extinction at 522 nm equals 2.2) and 500 μL of 3'-A$_{12}$Coc$_{Au}$ (extinction at 522 nm equals 2.2) were mixed in the presence of 300 mM NaCl, 25 mM Tris acetate buffer, pH 8.2, and 100 nM of the cocaine aptamer/extension (Cocaine Linker). The sample was warmed to 65° C for one minute and then allowed to cool slowly to

4°C. The nanoparticles changed color from red to dark purple during this process. The sample was centrifuged and the precipitates collected. The collected precipitates were then dispersed in 2000 μL of another buffer (150 mM NaCl, 25 mM Tris acetate, pH 8.2. The suspension was used for detection of cocaine.

[00103]    Example 8: Colorimetric Detection of Cocaine

[00104]    One-hundred microliters of the above prepared cocaine sensor suspension were combined with a small volume of concentrated cocaine solution. For example, 1 μL of 100 mM cocaine was added to the suspension to give a final concentration of 1 mM. The color change was monitored with a UV-vis spectrometer or by the naked eye.

[00105]    Example 9: Selectivity and Sensitivity of the Cocaine Sensor

[00106]    To a quarts UV-vis spectrophotometer cell (Hellma, Germany) was added 100 μL of the cocaine sensor system prepared in Example 7. A small volume (0.5-2 μL) of solutions including cocaine, adenosine, or sucrose was added to the spectrophotometer cell to bring the analyte concentration to the desired level in the cell.

[00107]    The dispersion kinetics for each cell was monitored as a function of time using a Hewlett-Packard 8453 spectrophotometer. FIG. 8A is a graph depicting the ratios of extinction at 522 and 700 nm plotted as a function of time. As may be seen from the plots, only cocaine gave a significant increase in the extinction ratio as a function of time, while adenosine and sucrose provided a color change consistent with the background. Therefore, the high selectivity of the sensor was confirmed.

[00108]     FIG. 8B is a graph depicting the correlation between the observed extinction ratios for the color change of the sensor system and the concentration of the cocaine analyte after one minute of aggregation.  The exceptional linearity of the sensor system was evident from about 0.1 to about 1 mM.

[00109]     FIG. 8C is a graph depicting the extinction ratios for multiple cocaine analyte concentrations over a 5 minute time period.  The graph demonstrates the ability of the sensor system to effectively differentiate between different analyte concentrations within a few minutes.  Thus, the ability of the sensor system to provide accurate quantitative information was established.

[00110]     In addition to the instrumental method of FIG. 8B, the color developed by the sensor was conveniently observed visually.  A color progression from blue to red was seen as the cocaine concentration increased from 0 to 1mM.  Adenosine and sucrose provided a color similar to the background.

[00111]     As any person of ordinary skill in the art will recognize from the provided description, figures, and examples, that modifications and changes can be made to the preferred embodiments of the invention without departing from the scope of the invention defined by the following claims and their equivalents.

**What is claimed is:**

1.      A sensor system for detecting an analyte, comprising:

a linker comprising an aptamer that folds in response to the analyte; and

second particles coupled to a second oligonucleotide, the second oligonucleotide complementary to at least a portion of the aptamer.

2.      The system of claim 1, where the linker comprises an extension.

3.      The system of any of the preceding claims, where the second oligonucleotide is complementary to at least a portion of the extension.

4.      The system of any of the preceding claims, where at least a portion of the bases forming the extension inhibit the linker from forming an inherently stable secondary structure without the analyte.

5.      The system of any of the preceding claims, further comprising first particles coupled to a first oligonucleotide, the first oligonucleotide complementary to at least a portion of the extension.

6.      The system of any of the preceding claims, where the extension comprises from 1 to 100 bases, at least 50 % of the bases complementary to the first or second oligonucleotide.

7.      The system of any of the preceding claims, where a portion of the first oligonucleotide is not complementary to the extension.

8.      The system of any of the preceding claims, where the hybridization stability of the aptamer in combination with the analyte is greater than the hybridization stability of a portion of the aptamer with the second oligonucleotide.

9.    The system of any of the preceding claims, where the first and second particles comprise a material selected from the group consisting of metals, semiconductors, magnetizable materials, and combinations thereof.

10.    The system of any of the preceding claims, where the first and second particles comprise gold.

11.    The system of any of the preceding claims, where the analyte is a metal ion.

12.    The system of any of the preceding claims, where the analyte is selected from the group consisting of large biomolecules, small biomolecules, and organic molecules.

13.    The system of any of the preceding claims, where the linker comprises a polynucleotide having a sequence selected from the group consisting of SEQ ID NOS. 46, 52, 54, and conservatively modified variants thereof.

14.    The system of any of the preceding claims, where the aptamer comprises a polynucleotide having a sequence selected from the group consisting of SEQ ID NOS. 1-42 and conservatively modified variants thereof.

15.    The system of any of the preceding claims, where
        the first oligonucleotide comprises a polynucleotide having a sequence comprising SEQ ID NO. 44 and conservatively modified variants thereof, and
        the second oligonucleotide comprises a polynucleotide having a sequence selected from the group consisting of SEQ ID NOS. 45, 53, 55, and conservatively modified variants thereof.

16.    The system of any of the preceding claims, where the linker comprises SEQ ID NO. 54, the first oligonucleotide comprises SEQ ID NO. 44, and the second oligonucleotide comprises SEQ ID NO. 55.

17.    The system of any of the preceding claims having a response time of less than 60 minutes.

18.    The system of any of the preceding claims having a response time of less than 10 minutes at from 23° to 37° C.

19.    A method of detecting an analyte, comprising:
        combining an aggregate with a sample; and
        detecting a color change responsive to the analyte, the aggregate comprising:
            a linker comprising an aptamer that folds in response to the analyte;
and
            second particles coupled to a second oligonucleotide, the second
oligonucleotide complementary to at least a portion of the aptamer.

20.    The method of any of the preceding claims, the aggregate further comprising first particles coupled to a first oligonucleotide, the first oligonucleotide complementary to at least a portion of an extension that forms a portion of the linker.

21.    The method of any of the preceding claims, where the second oligonucleotide is complementary to at least a portion of the extension.

22.    The method of any of the preceding claims, where at least a portion of the bases forming the extension inhibit the linker from forming an inherently stable secondary structure without the analyte.

23.    The method of any of the preceding claims, where the extension comprises from 1 to 100 bases, at least 50 % of the bases complementary to the first or second oligonucleotide.

24.    The method of any of the preceding claims, where the hybridization stability of the aptamer in combination with the analyte is greater than the hybridization stability of a portion of the aptamer with the second oligonucleotide.

25.    The method of any of the preceding claims, further comprising adjusting the ionic strength of the sample.

26.    The method of any of the preceding claims, where combining is selected from the group consisting of adding the sample to the aggregate and adding the aggregate to the sample.

27.    The method of any of the preceding claims, where the first and second particles comprise gold.

28.    The method of any of the preceding claims, where the analyte is selected from the group consisting of metal ions, large biomolecules, small biomolecules, and organic molecules.

29.    The method of any of the preceding claims, where the aptamer comprises a polynucleotide having a sequence selected from the group consisting of SEQ ID NOS. 1-42 and conservatively modified variants thereof.

30.    The method of any of the preceding claims, where
        the first oligonucleotide comprises a polynucleotide having a sequence comprising SEQ ID NO. 44 and conservatively modified variants thereof, and
        the second oligonucleotide comprises a polynucleotide having a sequence selected from the group consisting of SEQ ID NOS. 45, 53, 55, and conservatively modified variants thereof.

31.    The method of any of the preceding claims having a response time of less than 10 minutes at from 23° to 37° C.

32.    The method of any of the preceding claims, where the combining occurs from 20 to 30° C.

33.    The method of any of the preceding claims, where the aggregate disaggregates in response to the analyte.

34.    The method of any of the preceding claims, where the response is proportional to the quantity of the analyte in the sample.

35.    The method of any of the preceding claims, further comprising quantifying the color change.

36.    The method of any of the preceding claims, where the origin of the sample is selected from the group consisting of biological sources, industrial waste streams, and water supplies from which water is drawn for human consumption.

37.    A kit for the detection of an analyte, comprising:
        a system for forming aggregates, comprising:
                a linker comprising an aptamer that folds in response to the analyte;
and
                second particles coupled to a second oligonucleotide, the second oligonucleotide complementary to at least a portion of the aptamer;
        at least one first container containing the aggregate forming system, where a sample may be added to a container selected from the group comprising the first container and a second container.

38.    The kit of any of the preceding claims, where the hybridization stability of the aptamer in combination with the analyte is greater than the hybridization stability of a portion of the aptamer with the second oligonucleotide.

39.    The kit of any of the preceding claims, where the aptamer comprises a polynucleotide having a sequence selected from the group consisting of SEQ ID NOS. 1-42 and conservatively modified variants thereof.

40.    The kit of any of the preceding claims, further comprising instructions to form the aggregate.

41.    The kit of any of the preceding claims, the aggregate forming system further comprising first particles coupled to a first oligonucleotide, the first oligonucleotide complementary to at least a portion of an extension that forms a portion of the linker.

42.    The kit of any of the preceding claims, where the second oligonucleotide is complementary to at least a portion of the extension.

43.    The kit of any of the preceding claims, where the extension comprises from 1 to 100 bases, at least 50 % of the bases complementary to the first or second oligonucleotide.

44.    The kit of any of the preceding claims, further comprising a reagent to modify the ionic strength of the sample.

45.    The kit of any of the preceding claims, further comprising instructions to modify the ionic strength of the sample.

46.    The kit of any of the preceding claims, further comprising a reagent to modify the pH of the sample, the reagent selected from the group consisting of acids and bases.

47.    The kit of any of the preceding claims, further comprising a device to quantify a color change responsive to disaggregation of the aggregate.

48.     The kit of any of the preceding claims, where the device is selected from the group consisting of spectrophotometers and color comparators.

49.     The kit of any of the preceding claims, further comprising a light-down sensor system responsive to the analyte.

50.     A method for determining the sensitivity and selectivity of an aptamer to an analyte, comprising:

        combining an aggregate with the analyte;

        detecting a color change responsive to the analyte, the aggregate comprising:

                a linker comprising a DNA strand; and

                second particles coupled to a second oligonucleotide, the second oligonucleotide complementary to at least a portion of the DNA strand;

        determining if the DNA strand folded to provide the color change.

51.     The method of any of the preceding claims, the aggregate further comprising first particles coupled to a first oligonucleotide, the first oligonucleotide complementary to at least a portion of an extension that forms a portion of the linker.

52.     The method of any of the preceding claims, where the second oligonucleotide is complementary to at least a portion of the extension.

53.     The method of any of the preceding claims, where the extension comprises from 1 to 100 bases, at least 50 % of the bases complementary to the first or second oligonucleotide.
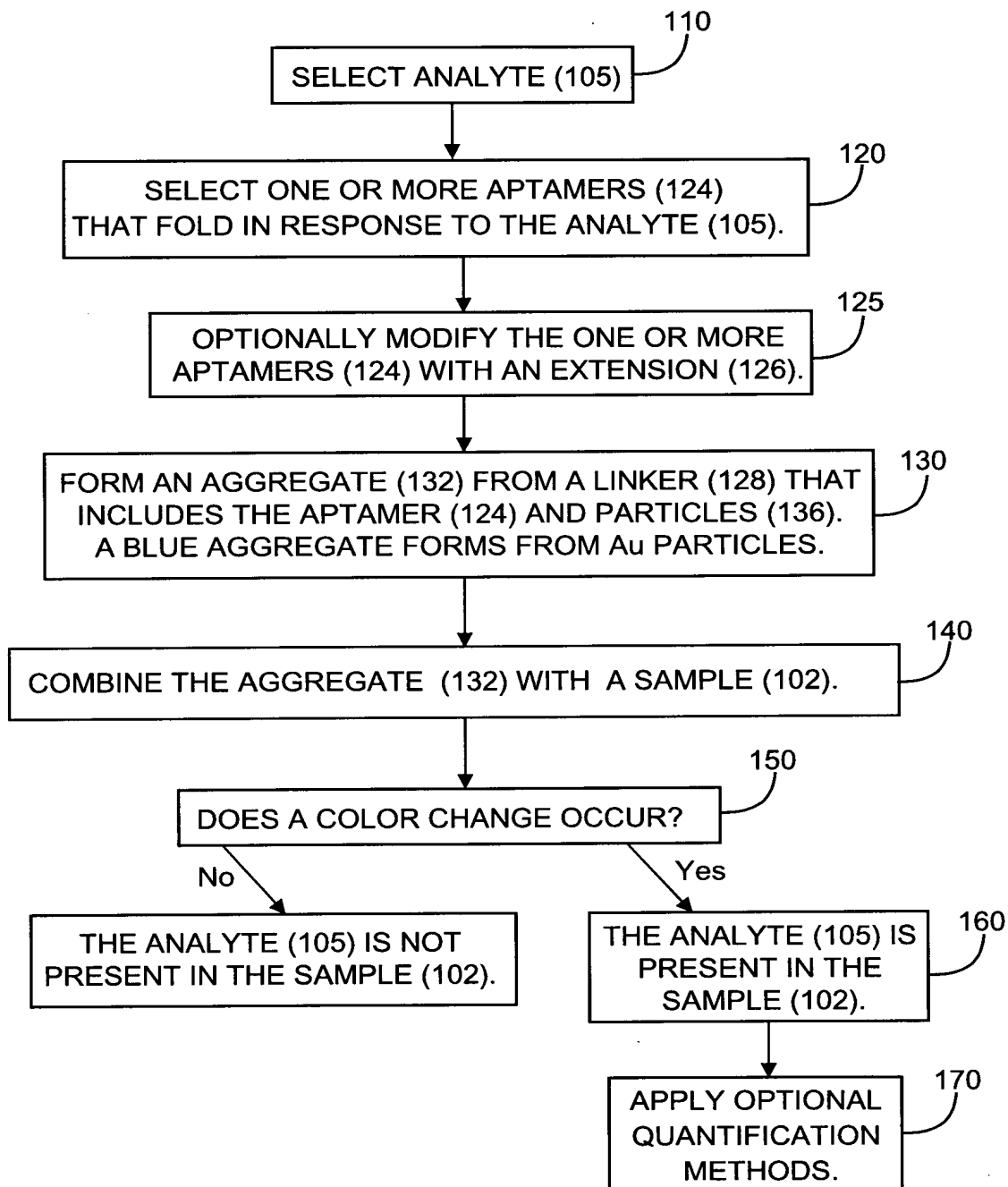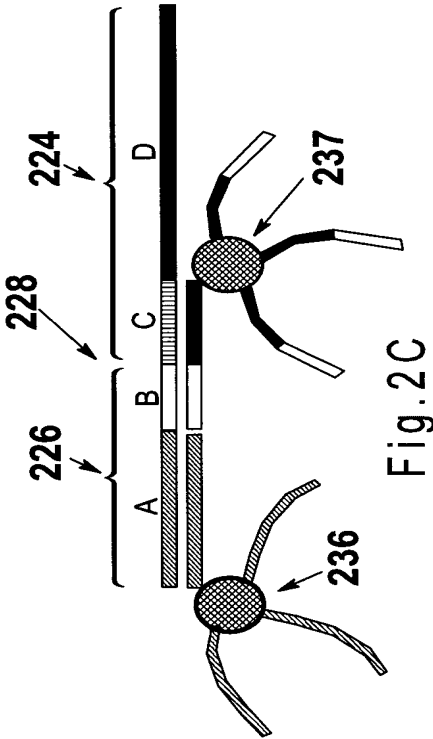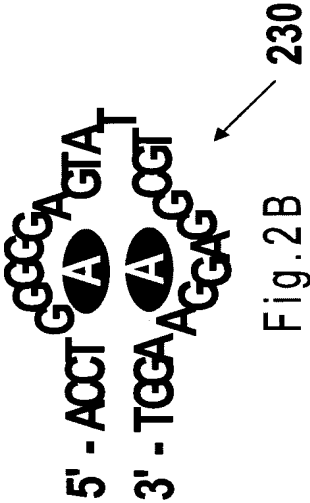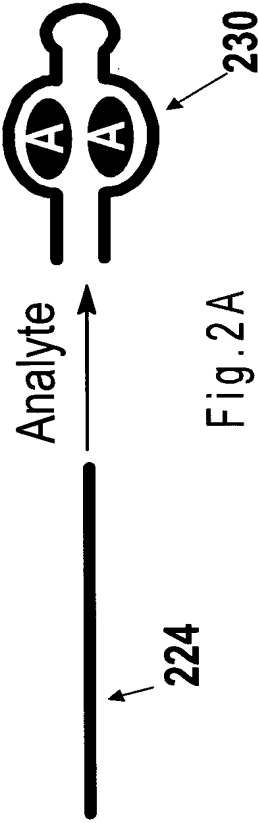
1/16

100



```
                            ┌──────────────────────────────┐ 110
                            │    SELECT ANALYTE (105)        │
                            └──────────────────────────────┘
                                          │
                                          ▼
               ┌────────────────────────────────────────────┐ 120
               │   SELECT ONE OR MORE APTAMERS (124)          │
               │ THAT FOLD IN RESPONSE TO THE ANALYTE (105).  │
               └────────────────────────────────────────────┘
                                          │
                                          ▼
               ┌────────────────────────────────────────────┐ 125
               │  OPTIONALLY MODIFY THE ONE OR MORE           │
               │  APTAMERS (124) WITH AN EXTENSION (126).     │
               └────────────────────────────────────────────┘
                                          │
                                          ▼
           ┌──────────────────────────────────────────────────┐ 130
           │ FORM AN AGGREGATE (132) FROM A LINKER (128) THAT   │
           │ INCLUDES THE APTAMER (124) AND PARTICLES (136).    │
           │ A BLUE AGGREGATE FORMS FROM Au PARTICLES.          │
           └──────────────────────────────────────────────────┘
                                          │
                                          ▼
           ┌──────────────────────────────────────────────────┐ 140
           │  COMBINE THE AGGREGATE  (132) WITH  A SAMPLE (102).│
           └──────────────────────────────────────────────────┘
                                          │
                                          ▼
                    ┌──────────────────────────────────┐ 150
                    │  DOES A COLOR CHANGE OCCUR?        │
                    └──────────────────────────────────┘
                     │ No                          │ Yes
                     ▼                             ▼
       ┌───────────────────────────┐   ┌─────────────────────────┐ 160
       │  THE ANALYTE (105) IS NOT  │   │  THE ANALYTE (105) IS    │
       │ PRESENT IN THE SAMPLE (102)│   │    PRESENT IN THE        │
       └───────────────────────────┘   │    SAMPLE (102).         │
                                        └─────────────────────────┘
                                                   │
                                                   ▼
                                        ┌─────────────────────────┐ 170
                                        │  APPLY OPTIONAL          │
                                        │  QUANTIFICATION          │
                                        │  METHODS.                │
                                        └─────────────────────────┘
```

Fig.1

Fig.2A

Fig.2B

Fig.2C

Fig.3A

395

No adenosine

332

Adenosine 305

337

337

390

Aggregate

337

336

331

328

326

324

328

5'

3'

ACTCATTCTGTGAAGAGAAACCTCCCCCAGTATTCCGGACGAAGGT

= 3' - TCTCTTCGGACGC 5' - (CH$_2$)$_6$- S - 

= 5'Ade$_{Au}$

= -S - (CH$_2$)$_6$- 3' AAAAAAAAAAATGAGTAGACACT- 5' = 3'A$_{12}$Ade$_{Au}$

336

Fig.3B

Fig.3C

Fig.3D

Fig.4

Fig.5A

Fig.5B

Fig. 5C

Fig.6A

Fig.6B

Fig.6C

Fig.7A

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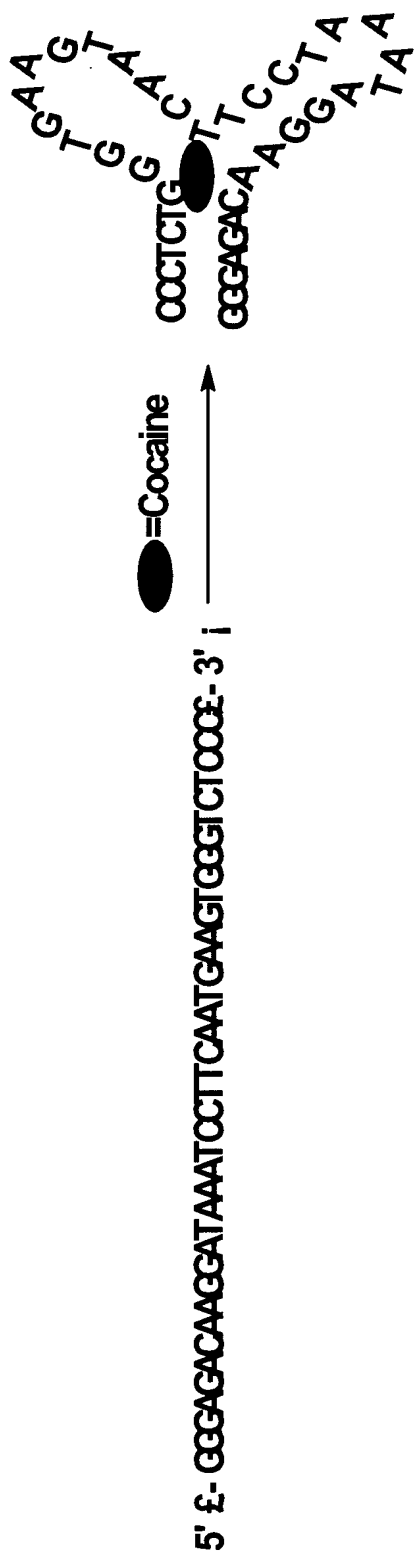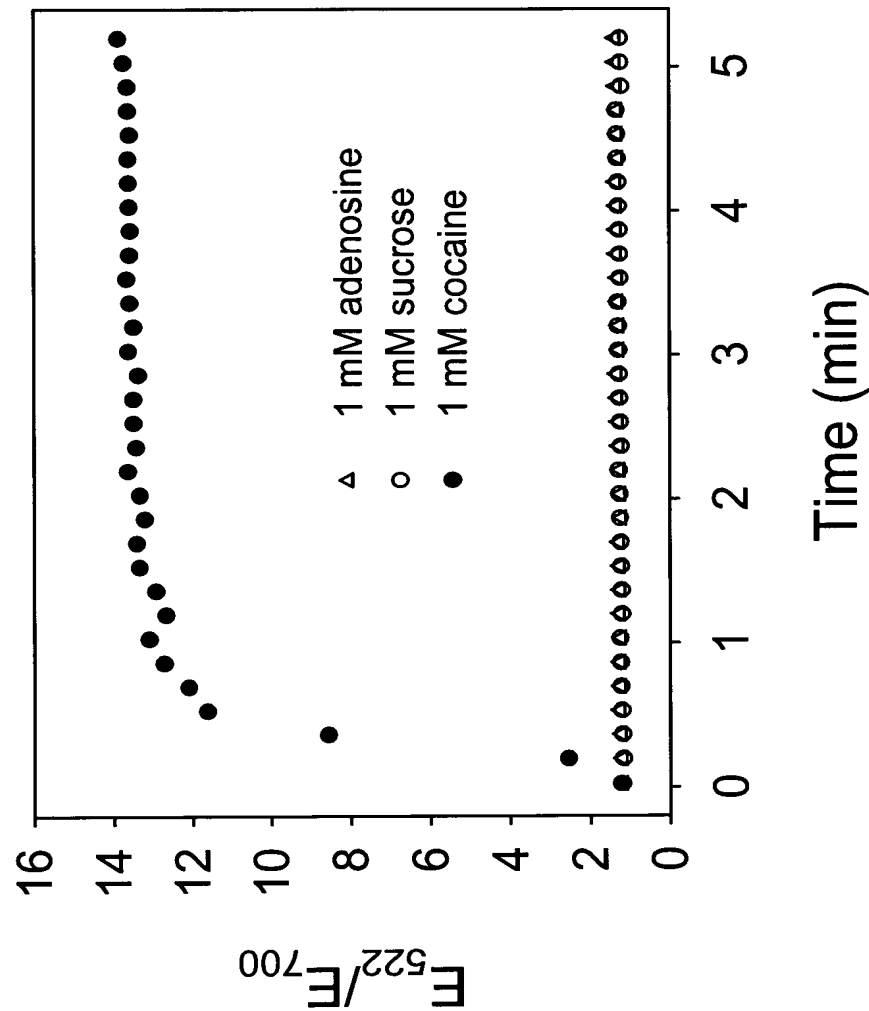


Fig. 7B

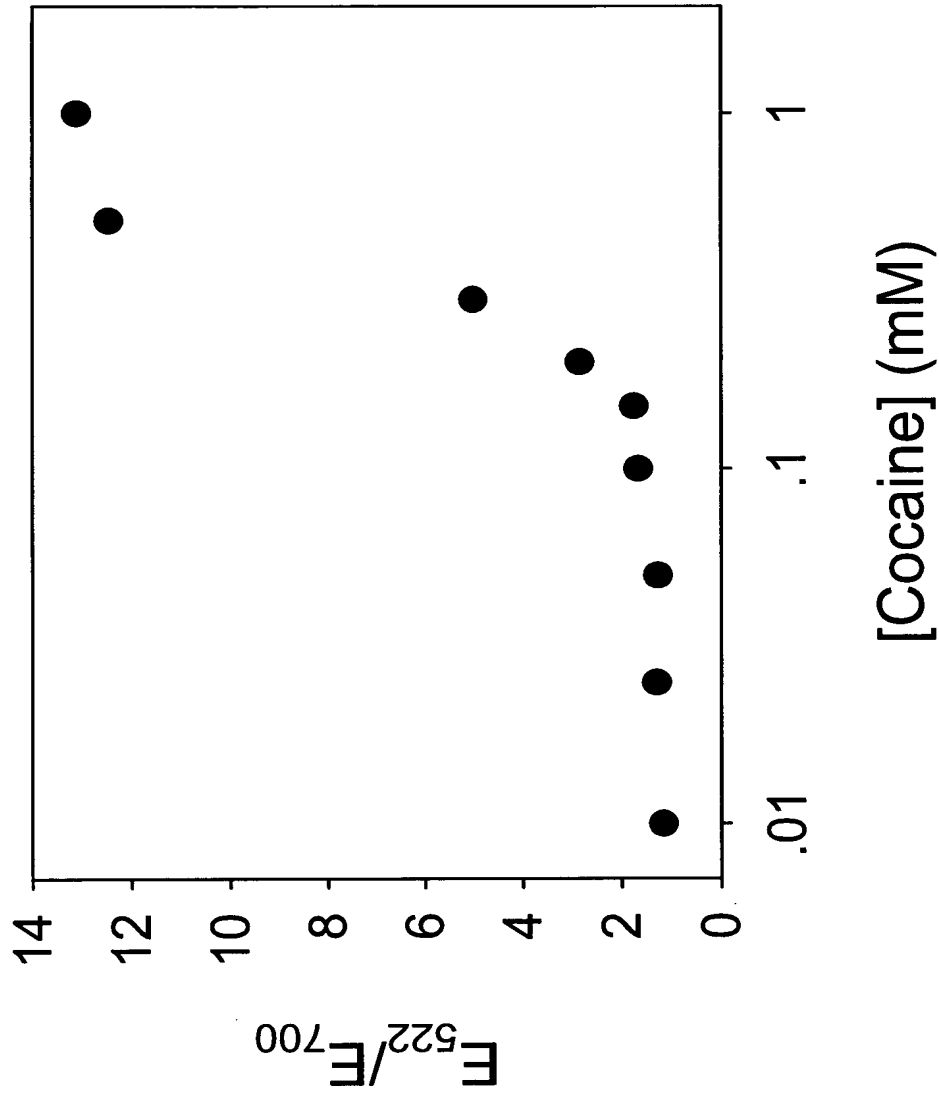Fig.8A

Fig.8B

Fig.8C

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<130> ILL01-076-US

<140> 11/202,380
<141> 2005-08-11

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<210> 22
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<210> 29
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<210> 33
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<400> 34
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<210> 35
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<210> 42
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<211> 28
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<220>
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<210> 46
<211> 44
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<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide

<400> 46
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<210> 47
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<211> 17
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<220>
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<400> 47
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<210> 48
<211> 7
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<220>
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      oligonucleotide

<400> 48
acctggg                                                               7


<210> 49
<211> 7
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<220>
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<400> 49
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<210> 50
<211> 44
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<220>
<223> Description of Artificial Sequence: Synthetic
      linker

<400> 50
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<210> 51
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<220>
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<400> 51
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actcatctgt gaagagaacc tggggagta ttgcggagga aggt                          44


<210> 52
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<220>
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<400> 52
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<210> 53
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<400> 53
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<210> 54
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<400> 54
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<210> 55
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide

<400> 55
gtctcccgag a                                                            11


<210> 56
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<220>

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<400> 56
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<210> 57
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<220>
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<400> 57
acctggggga gtattgcgga ggaaggt                                          27


<210> 58
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide

<400> 58
gtctcccgag at                                                          12


<210> 59
<211> 39
<212> DNA
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<220>
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<400> 59
gggagacaag gataaatcct tcaatgaagt gggtctccc                              39
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